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Size-Structure Correlation in Developing Roots of Cissus and Syngonium

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ABSTRACT — The development of conducting tissues in relation to root size is examined in *Cissus sicyoides* L. and *Syngonium podophyllum* Engl. Evidence is presented that such relationships arise earlier in development than has hitherto been described. Their early appearance calls into question the hypothesis that such relationships reflect a solution to problems of water economy in mature tissues. While pattern determination in *Syngonium* is similar to that anticipated on the basis of published investigations, a new kind of size-structure correlation is identified in *Cissus*. In light of this fact, a need to broaden the philosophical bases for an interpretation of form in relation to size is suggested.

It has become commonplace to observe a correlation in vascular plants between the amount of conducting tissue in a mature plant structure and the size of that structure. It has become commonplace as well to demonstrate this correlation in roots (in stems, a relationship between the development of vascular tissues and axis size may be obscured by the influence of leaves or other lateral appendages). A final commonplace is the mechanism of adjustment encountered in those roots with a pith: for a given species, the number of units comprising the vascular system is roughly predictable from root diameter. The historical ascendance of these generalities, insofar as they relate to the present study, may be outlined briefly.

Relationships between size and structure, recognized at least since the time of de Bary (1884), were first systematically investigated by Wardlaw (1924, 1925, 1928) and Bower (1921, 1923, 1930). Working entirely with mature tissues, they described the increase in extent and elaboration of the xylem concomitant with increasing bulk of the organ traversed by this tissue. Bower (1930) first emphasized the utility of exploring such relationships in roots: "The fact that gives the conducting tract of the root a special value . . . lies in the absence of appendicular organs . . . Accordingly we may see in the stele of the root an opportunity for the study of the relation of size to structure naturally simpler and more likely to yield direct results than in that of the leafy shoot." In summarizing his investigations on this aspect of root organization (1930), he stated: "The most constant fact is that the stelar structure of roots is of the type styled 'radial,' with the protoxylem seated at the extremity of each ray. The number of rays may vary from one to very high figures, and the evidence shows that there is a near relation of their number to the size of the stele, though no exact numerical ratio exists." Wardlaw (1928), in a discussion of factors influencing root morphology, first called attention to the lack of information about how such correlations arise during devolopment. Turing (1952) proposed an elegant mathematical model accounting for a relationship between organ size and the number of vascular units differentiated during development. He suggested that such a model would be most closely approximated by the development of the vascular pattern in roots with a pith. In experimental work by Bünning (1952) on *Vicia faba*, 2 millimeter apical segments of roots were severed, slightly rotated and replaced, and discontinuous steles resulted. He concluded that pattern determination occurs early in ontogeny, probably in response to factors indigenous to the apical meristem or its immediate derivatives. Attention thus focused increasingly upon analyses of size-structure correlations during their development. In recent studies, protoxylem (Torrey, 1955) and protophloem (Cahoon, 1957) have served as parameters for investigating pattern determination in roots. In each of these studies, a clear correlation between the size of the meristematic stele and the number of developing vascular units was observed. Evidence from theoretical, experimental and descriptive studies thus reinforced the long-standing impression that variation in root size is paralleled by variation in the number of units comprising the vascular system, as well as indicating that this relationship is established well before the maturation of the bulk of the conducting tissues.

Protophloem elements are ordinarily the first representatives of the vascular tissues to mature. At stages in development prior to their differentiation, comparisons between the developing stele (all tissues bounded by the prospective endodermis) and the balance of the meristematic system might well reveal still earlier indications of the size-structure correlation. In the course of the present investigation, initiated to explore this possibility, it was noted that, in *Cissus sicyoides* L., the anticipated "near relation" between the number of vascular units and stele size was not at all apparent. In *Syngonium podophyllum* Engl., another species investigated, this aspect of the size-structure correlation was readily observable. For this reason, a detailed comparison of pattern determination in the two species was undertaken.

Collection and fixing of roots

Adventitious roots of *Cissus sicyoides* L. (Vitaceae) and *Syngonium podophyllum* Engl. (Araceae) were collected and fixed immediately in Randolph's modified Navashin Fluid (Randolph, 1935). Following dehydration in an ethyl-butyl alcohol series, the material was infiltrated with paraffin, embedded, serially sectioned and stained according to one of the following schedules: safranin – fast green or tannic acid – iron chloride followed by safranin – aniline blue (Johansen, 1940).

Outline drawings of each section to be measured were

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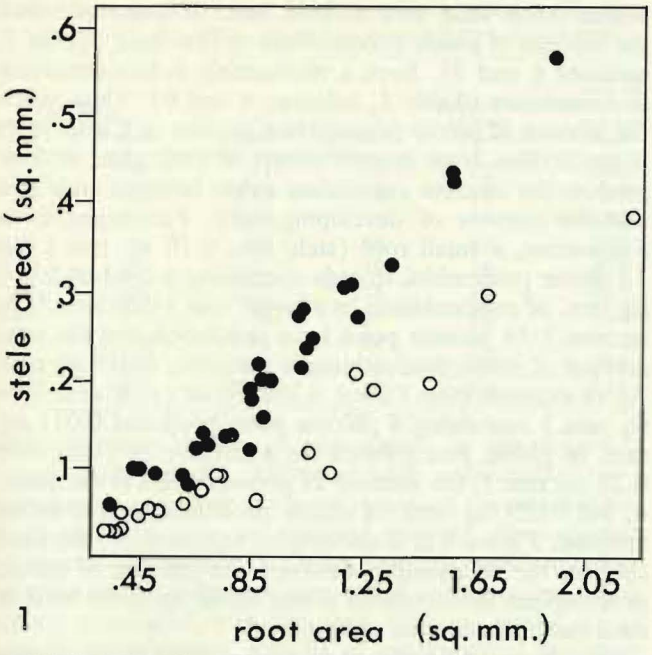


Figure 1

made using a Wild microscope, model M20, with drawing-tube attachment. The sections were traced using the oil-immersion lens system with a total magnification of 1000X. Area measurements of the tracings were made using a Filotechnica Salmoiraghi planimeter, model 236A, and appropriately converted. Measurements were recorded for ten roots of each species.

Cross-section analysis

For each species, the selection of cross-sections to be measured was based upon easily identifiable ontogenetic changes during tissue differentiation. The use of such selection criteria permitted an analysis of those cross-sections representing nearly equivalent stages in development among all roots in a species sample.

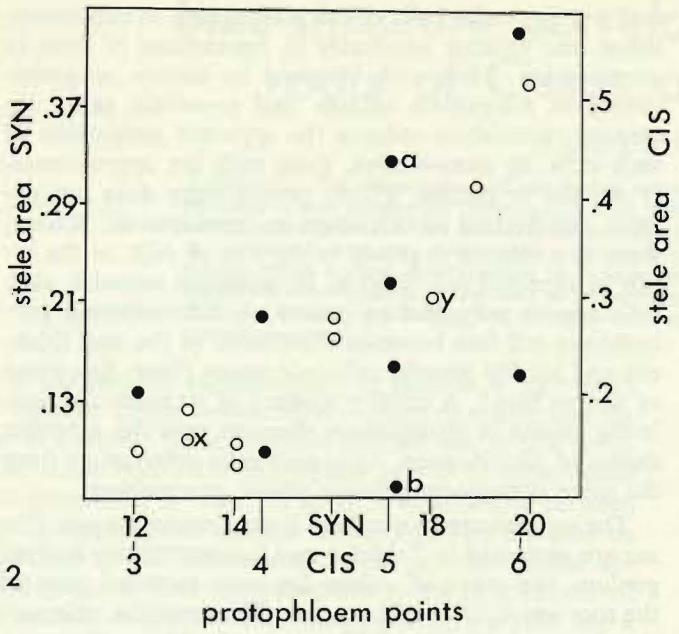
Three such changes can be identified with particular ease in *Cissus* roots. *Level one.* Roughly one-half millimeter behind the root apex (exclusive of the root cap), the propective endodermis begins to accumulate compounds which appear a bright yellow in sectioned material; the meristematic central cylinder, as a result, is clearly distinguishable. *Level two.* About six-tenths of a millimeter behind the apex, raphide sacs differentiate from the ground meristem of the prospective cortex. *Level three.* Approximately eight-tenths of a millimeter behind the apex, the first protophloem element matures within each strand of phloic procambium.

Measurements were recorded from two levels in each *Syngonium* root. *Level one.* About six-tenths of a millimeter behind the apex, the radial arrangement of ground meristem cells bordering the prospective endodermis clearly delimits the boundary of the meristematic stele. *Level two.* Roughly one millimeter behind the apex, the first protophloem element matures within each strand of procambium.

Tissue areas within particular cross-sections were delimited on the basis of the following criteria. The stele includes all tissues bounded by the prospective endodermis. Prospective phloem areas within the stele can be identified readily on the basis of cell size, degree of cell vacuolation and relative angularity in appearance of cells in cross-section. Most cells destined to mature as parenchyma or metaxylem enlarge and vacuolate early; increased vacuolation reduces the apparent stainability of such cells. In cross-section, these cells are approximately circular in outline. Phloic procambium does not enlarge significantly at this stage in development. Rather, there is a relative decrease in the size of cells as the result of repeated cell division. In sectioned material, such cells appear polygonal in outline. A differentiating protophloem cell first becomes identifiable as the wall thickens and readily absorbs cellulosic stains (here, fast green or aniline blue). A relative absence of stainable contents in the lumina of protophloem elements provides a further means of identification, since such cells differentiate from the more densely-cytoplasmic phloic procambium.

The measurements obtained from *Syngonium* and *Cissus* are presented in Tables 1 and 2, respectively. In *Syngonium,* the range of values for cross-sectional area of the root was 0.37 – 2.21 sq. mm. The range for stele sectional area was 0.05 – 0.39 sq. mm. In *Cissus,* the range of values for total root area was 0.32 – 1.93 sq. mm.; that for stele area, 0.03 – 0.57 sq. mm. Figure 1 is a scatter-plot representing the relationship, for each species, between stele area and total root area at all levels analyzed. Each root of *Cissus* is thus represented by three points in the scatter-plot; each root of *Syngonium,* by two points. The scatter-plot reveals a clear correlation between the area of the developing stele and the total area of the yet-meristematic root. Such a correlation represents an expression of the size-structure relationship earlier in development than has hitherto been described. In this instance, form is related to size at the moment of its morphological inception, well before the maturation of any of the vascular tissues, preceding, in these species, even procambial differentiation.

The number of phloem poles established at the oldest analyzed level in each root (for both species, that level at which at least one protophloem element had matured in in each phloic procambial strand) also was determined. A correlation between number of phloem poles and area of the meristematic stele was readily observable in *Syngonium* (Table 1, columns 4 and 5) but absent in *Cissus,* (Table 2, columns 6 and 7). As compared with *Cissus,* roots in the *Syngonium* sample showed somewhat less variation in stele size but a greater range in the number of phloic procambial strands differentiated. In one of the smallest *Syngonium* roots (stele area 0.09 sq. mm.), 12 phloem poles developed, while in the largest (stele area 0.39 sq. mm.) 20 phloem poles had been produced. In contrast, the smallest *Cissus* root (stele area 0.10 sq. mm.) contained 5 phloem poles, the same number present in one of the largest (stele area 0.44 sq. mm.). A *Cissus* root with stele area (0.20 sq. mm.) twice that of the smallest root in the sample contained only 3 phloem poles, while one very slightly larger (stele area 0.22 sq. mm.) contained 6. In contrast, *Syngonium* roots with stele areas of 0.10, 0.20 and 0.30 sq. mm. produced 13, 16 and 19 phloem poles, respectively. Figure 2 is a



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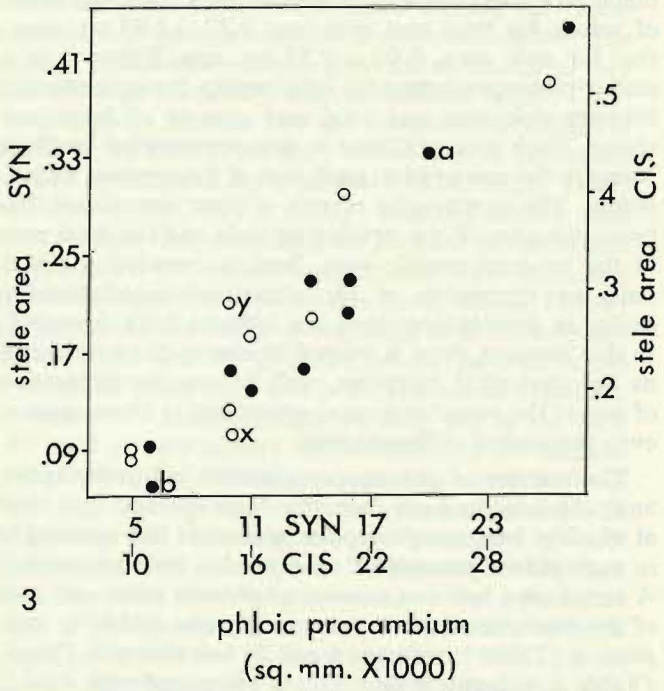

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scatter-plot representing the relationship between the number of phloic procambial strands differentiated at the oldest analyzed level in each root and the area of the stele at that level.

Since the number of phloic procambial strands differentiated in roots of *Cissus* seemed somewhat independent of stele size, a correlation involving the amount of phloic procambium produced seemed likely. For that reason, the total cross-sectional area of phloic procambium present at level three in each root was determined (Table 2, column 8). For purposes of camparison, similar measurements were recorded from the morphologically equivalent level, two, in each root of *Syngonium* (Table 1, column 6).

In *Cissus,* a high degree of correlation is indeed revealed when stele area at level three is compared with the amount of phloic procambium at that level (Table 2, columns 6 and 8). Such a relationship is less consistent in *Syngonium* (Table 1, columns 4 and 6). Thus, while the amount of phloic procambium present in *Cissus* roots is predictable from measurements of stele size, in *Syngonium* the clearest correlation exists between stele size and the number of developing units. For example, in *Syngonium,* a small root (stele area 0.10 sq. mm.) has 13 phloic procambial strands containing a total of 0.010 sq. mm. of procambium. In a larger root (stele area 0.21 sq. mm.) 18 phloem poles have developed, but the total amount of phloic procambium is the same, 0.010 sq. mm. As an example from *Cissus,* a small root (stele area 0.14 sq. mm.) containing 4 phloem poles produced 0.011 sq. mm. of phloic procambium. In a larger root (stele area 0.28 sq. mm.), the number of phloem poles is the same, 4, but 0.021 sq. mm. of phloic procambium had differentiated. Figure 3 is a scatter-plot representing, for each species, the relationship between the amount of phloic procambium differentiated at the oldest analyzed level in each root and stele size at that level.

In order to summarize these observations on alternative solutions to structure adjustment in relation to size, attention is called to points "a", "b", "x" and "y" in Fig. 2-3. In observing the relative positions of these points in the two scatter-plots, it becomes apparent that, in *Cissus* (points "a" and "b"), the size of the stele is closely correlated with the amount of developing phloic procambium, while in *Syngonium* (points "x" and "y") variation in stele size is reflected most clearly by variation in the number of developing provascular units.

Reason for the Correlation

In attempting an explanation for size-structure phenomena in plants, Wardlaw and Bower emphasized the functional value of elaborating the vascular pattern in response to increasing organ size. Such elaboration would tend to maintain surface-of-contact relationships between the conducting tissues and the structure in which they are embedded. Wardlaw (1928) stated: "the passage of water in and out of the conduits . . . appears to depend on the activity of the living cells abutting on the xylem. . . . Since the manipulation of the water supply is such a vital process in plant economy, it is clear that a large surface of contact between xylem and living parenchyma is not only an advantage but a necessity." Bower (1930) similarly suggested that water movement through the vascular tissues of the root "depends on the maintenance of a relatively large surface of contact between the osmotically active living cells and the dead tracheal elements."

Subsequent research has revealed that correlations between size and structure are apparent in meristematic tissues, and the "water economy" hypothesis of Wardlaw and Bower should perhaps be re-evaluated in light of such evidence. Subapical tissues which first reflect morphologically the inception of size-structure patterns are composed entirely of living cells. Thus surface-of-contact relationships, whatever their physiological significance at maturity, may be determined by interactions in a mani-

TABLE 1. *Syngonium*. Area measurements in square millimeters

LEVEL 1		LEVEL 2			
root	stele	root	stele	number of phloem poles	sectional area of phloic procambium
.37	.05	.71	.09	12	.005
.36	.03	1.12	.10	13	.010
.37	.03	1.03	.12	13	.010
.30	.03	.66	.08	14	.005
.32	.03	.73	.10	14	.010
.49	.04	1.27	.18	16	.011
.44	.05	1.49	.20	16	.014
.61	.06	1.21	.21	18	.010
.47	.06	1.68	.30	19	.016
.86	.07	2.21	.39	20	0.26

TABLE 2. *Cissus*. Area measurements in square millimeters

LEVEL 1		LEVEL 2		LEVEL 3			
root	stele	root	stele	root	stele	number of phloem poles	sectional area of phloic procambium
.32	.06	.42	.10	.42	.10	5	.011
.50	.09	.64	.12	.66	.14	4	.011
.77	.14	.88	.20	1.01	.22	6	.015
.61	.08	.83	.12	.91	.20	3	.016
.58	.09	.83	.18	.86	.22	5	.019
.89	.16	1.02	.27	1.21	.31	5	.019
.70	.13	.83	.19	1.03	.28	4	.021
.76	.14	1.07	.25	1.17	.31	5	.021
1.04	.24	1.35	.33	1.57	.44	5	.025
1.22	.27	1.57	.43	1.93	.57	6	.032

festly different theater of expression. In this regard, a recent study by this author of such relationships in a greenhouse specimen of *Cissus sicyoides*, the adventitious roots of which may be collected growing above ground or beneath the surface of a pool, failed to reveal any modification in the early development of the vascular pattern clearly associated with the shift from an aerial to an aquatic environment. Results of experimental investigations by Bünning (1952) on *Vicia faba* seem to indicate that, although factors regulating maturation may well diffuse towards the apex from mature tissues, factors controlling pattern determination are more probably direct constituents of the apical system. Luxova and Kozinka (1970) measured water flow through 5 mm. segments from primary seminal roots and adventitious roots of the first through fifth nodes in *Zea mays* L. They concluded that conductivity was not closely correlated to transectional area of the xylem in this system. Observations that (1) conductivity is poorly correlated with transectional area of the vascular pattern; (2) pattern determination seems to be a result of interactions in subapical regions; (3) such interactions do not result in consistent pattern differences when operating in two disparate environments; suggest, in some systems, that pattern determination is linked only indirectly to the solution of water economy problems at maturity. Correlations between size and structure may be established in response to factors quite different from those tending to maximize surface-of-contact relationships, or even conduction rate, in older tissues.

In recent analyses of pattern determination in roots (Torrey, 1955; Cahoon, 1957), strong correlations between the number of developing vascular units and the radial dimensions of the maturing system were identified. A similar relationship was observed in *Syngonium* roots in the present study. In *Cissus* roots, however, evidence for a correlation between size of the developing root and number of provascular units produced has not been demonstrated. Rather, a relationship is apparent between root size and the total sectional area of maturing phloic procambium. It is possible that factors controlling pattern determination in *Cissus* are different from those operating in *Syngonium* and other species. By the time of maturation of first protophloem elements in *Cissus*, size-adjustment mechanisms operate by regulating the relative development of procambial tissue at each phloem pole. The number of such poles to be established is determined, any relationship between this feature of the vascular pattern and root size is obscured (if it ever existed) early in development. By shifting the focus of attention from number of units to size of units, evidence from *Cissus* has added another dimension to the frame of reference within which the relationship between size and pattern determination must be considered.

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